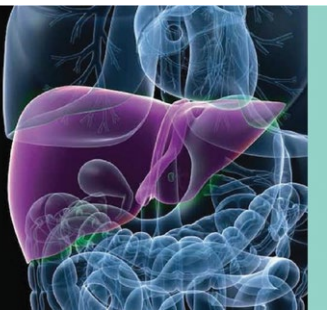

Hepatitis C Virus Diagnostics: The Road to Simplification

Jordan J. Feld, M.D., M.P.H.

The ambitious goal of eliminating viral hepatitis as a public health problem by 2030 will require major efforts to prevent new infections, but perhaps more importantly, there will have to be a dramatic increase in diagnosing those already infected and linking them to care. Although the algorithm to make a diagnosis of hepatitis C virus (HCV) infection may seem simple to the experienced clinician, the need for multiple tests, some with slow turnaround times, is proving to be a major impediment to achieving elimination targets.

The traditional approach to HCV diagnosis requires an initial antibody (Ab) test to document exposure, followed by a confirmation of ongoing viremia, usually using a polymerase chain reaction (PCR)–based assay to quantify the level of HCV RNA in the blood. Individuals who test Ab positive but HCV RNA negative have either spontaneously cleared the infection (usually within 6-12 months of initial infection) or been successfully treated. Other much less common possibilities are false-positive Ab tests or acute infection during which the HCV RNA can be transiently undetectable. Current Ab and HCV RNA assays have very high sensitivity and specificity, making false-positive and false-negative results rare occurrences.[1] The real challenge of the traditional approach is the need for two blood tests (Fig. 1). Although this may not seem so difficult, when one walks through the journey for a patient, it quickly becomes apparent that there are many places to get lost along the way (Fig. 2). The individual must first see a provider who recognizes the risk for HCV infection and orders the initial Ab test. A trip to the phlebotomist to get the test done is then required, followed by a return clinic visit for the result. If positive, a second requisition for HCV RNA is sent, requiring a return trip to the laboratory and a return trip to the clinic for the result. If any one of the steps along the way to a confirmed diagnosis is missed, the individual may never know of the infection, leading to a missed opportunity for treatment and possibly a risk for inadvertent ongoing transmission. The many steps in the process

Abbreviations: Ab, antibody; Ag, antigen; DAA, direct-acting antiviral; DBS, dried blood spot; F/U, follow-up; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; PCR, polymerase chain reaction; POC, point-of-care; SVR, sustained virological response.

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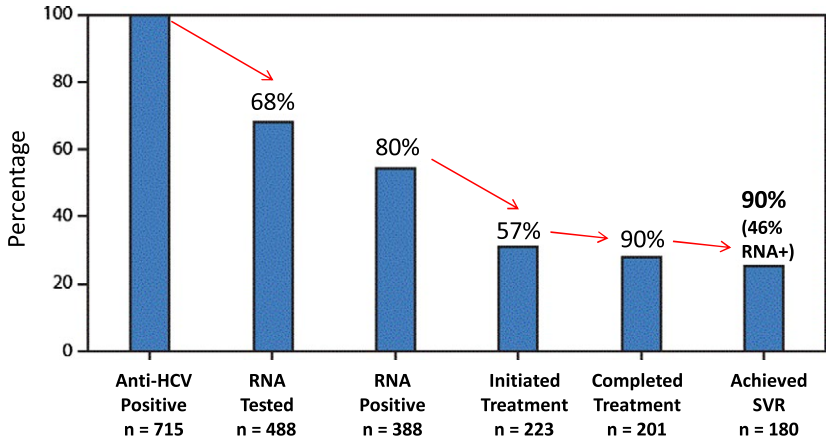

FIG 1 Cascade with drop-off to HCV RNA. As shown, one of the largest drop-offs in the cascade of care occurs after HCV antibody testing. Many patients never receive an HCV RNA test to determine whether they have active infection and require therapy. Reprinted with permission from Mera et al. 2016.[3]

disproportionally limit diagnosis in marginalized populations, many of which have high burdens of HCV infection.[2] Challenges of the two-test approach for specific populations are highlighted in Table 1, ranging from the need for venipuncture to the requirement to see people on multiple occasions.

SIMPLIFICATION USING CURRENT TESTS

The simplest solution to ensuring an HCV RNA test is done for all positive Ab tests is to reflex the testing. PCR can be performed on leftover serum after the Ab test; however, there are some issues to consider. RNA that is

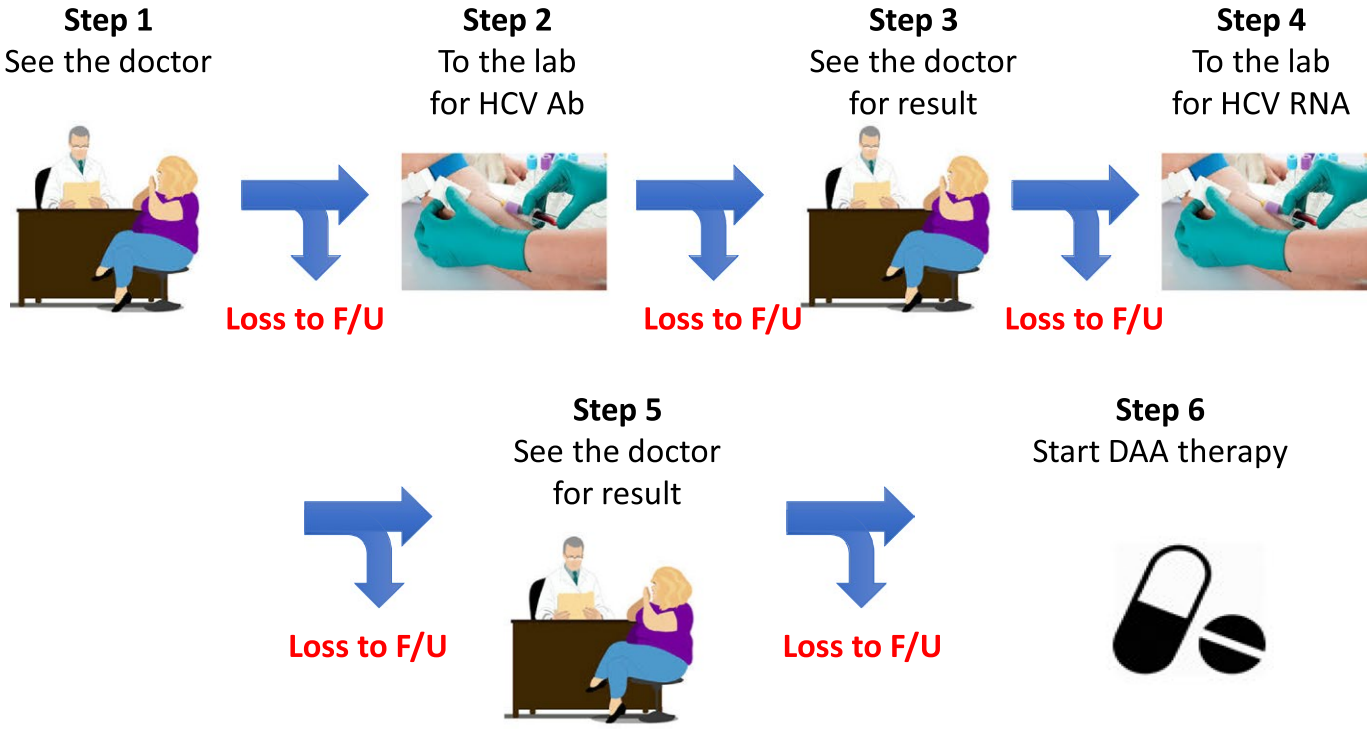

FIG 2 Steps in current algorithm for HCV diagnosis. Each of the multiple steps, from initial testing to a confirmed diagnosis of chronic HCV infection, leave the potential for loss to follow-up (F/U).

TABLE 1. SOME CHALLENGES OF STANDARD TWO-TEST MODEL FOR HCV DIAGNOSIS IN DIFFERENT POPULATIONS

Population	Challenges
People who inject drugs	• Need for venipuncture • Multiple visits required for diagnosis
Incarcerated individuals	• Need for venipuncture—trained staff • Delay in diagnosis; rapid movement within facilities—test results may not follow individual • Poor linkage of results for follow-up after release
Rural/remote	• Multiple visits required for diagnosis • Need for venipuncture—trained staff
Poor, uninsured/underinsured, unstable housing	• Multiple visits required for diagnosis • May not have facilities to be contacted for results (e.g., phone, address)
Newcomers/immigrants	• Multiple visits required for diagnosis • Language barriers may lead to miscommunication about need for follow-up

improperly stored may degrade. Fortunately, accurate quantification is rarely relevant with current therapy, and there is a low probability of a positive sample degrading sufficiently to become negative.[4] To minimize degradation, if a single tube is collected that will be used for Ab and RNA testing, the collection times must be done as if for RNA, and the residual serum must be correctly stored while awaiting Ab results. This requires coordination if Ab and RNA testing are done in different laboratories or even different sections of the same laboratory. However, the ability of large commercial laboratories to operationalize this process demonstrates that it can be done effectively, with results showing near-universal completion of an RNA test on all Ab-positive results. Collecting two tubes at baseline is also possible but adds significant cost when one considers the amount of screening required worldwide.

DRIED BLOOD SPOT TESTING

Another mechanism for enabling reflex testing is the use of dried blood spot (DBS) testing for HCV. The approach has been used in other fields, particularly human immunodeficiency virus (HIV), with great success.[5] A fingerprick of whole blood is applied to a card with four or five individual filter paper spots (Fig. 3). After drying, the blood remains very stable on the DBS card, allowing for simple shipping, even using regular mail. Once in the laboratory, the serum is eluted off the card and can be run using the standard automated serum detection systems. The first spot is tested for Ab, and if positive, the next spot is tested for HCV RNA.[6] Because of the lower quantity of blood, the HCV RNA loads from DBS are lower than in a serum

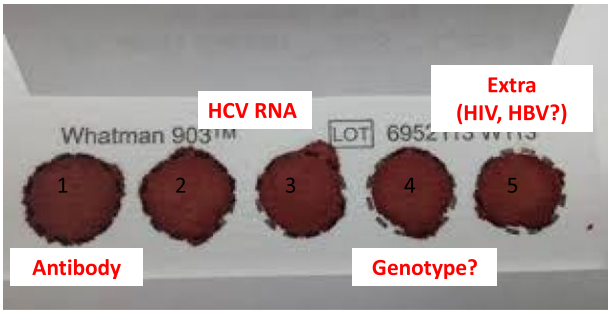

FIG 3 DBS testing allows for sequential testing to occur. Whole blood is taken from a fingerprick, meaning venipuncture is not required. After drying, the card can be sent to the central laboratory for testing. The first spot can be tested for HCV antibody, and if positive, subsequent spots can be tested for HCV RNA, HCV genotype, and other infections.

tube from the same patient by ~2 log IU/mL.[7] This is important if results are used for quantification, such as deciding whether to shorten therapy with sofosbuvir/ledipasvir, but is otherwise rarely clinically relevant because more than 95% of individuals with chronic infection have HCV RNA levels greater than 4 log IU/mL, making it extremely unlikely to go from positive in serum to negative on DBS.[7] Another advantage is the lack of requirement for venipuncture, which is very helpful for people with a history of injection drug use and also enables testing to be done by individuals with minimal training, including peer workers. Also, other spots can be used for other serological tests (e.g., HIV, hepatitis B virus [HBV], and syphilis). Although potentially useful, particularly in rural and remote settings, DBS is not faster than standard serum testing and may affect workflow in the laboratory because of the requirement for an elution step.

POINT-OF-CARE AND RAPID DIAGNOSTIC TESTS

The ability to obtain an immediate test result avoids the need for return visits and tracking down individuals who do not return. Point-of-care (POC) tests have been developed for HCV Ab and, more recently, for HCV RNA. POC Ab tests are particularly useful in settings where follow-up may be challenging, such as screening drives or testing in emergency departments. The approved OraQuick test requires only a fingerprick and provides a result in 20 minutes, allowing for those testing positive to have an immediate follow-up test for HCV RNA. A version of the test using salivary fluid has been developed but has slightly lower sensitivity and was therefore not approved in North America.[8] The option to avoid drawing blood at all may be a major advantage in certain settings and countries. Weighing the reduced sensitivity against increased testing uptake should be considered with considerations of the overall cost-effectiveness, which may favor this approach in many settings. Other POC Ab tests have also been developed.

More recently, a rapid HCV RNA test has been Conformité Européene (CE)-marked in Europe. HCV RNA quantification can be done on the GeneXpert using a drop of whole blood or serum with a turnaround time of approximately 90 to 100 minutes. Individual cartridges for blood collection are placed into a module that does automated nucleic acid extraction and amplification.[9] The test is not yet approved in North America but may be useful in many regions of the world that use the same platform for rapid diagnosis of other infections, such as tuberculosis and HIV, and thus already have the modules available in testing facilities. In very high prevalence settings (e.g., prisons, opioid substitution clinics), it may be reasonable to consider going directly to HCV RNA testing without initial Ab testing. The major advantage would be reducing the time for a confirmed diagnosis, which currently, even using rapid Ab and RNA testing, likely requires close to 2 hours. The success of direct-acting antivirals for HCV means that on-treatment monitoring is rarely required, which may allow for tests to move to qualitative (positive/negative) strategies with somewhat reduced sensitivity, that will hopefully improve the turnaround time for HCV RNA detection to make it truly POC.[10]

OTHER TESTS: CORE ANTIGEN

The purpose of HCV RNA testing is to confirm viremia. Detection of the core antigen (Ag), a part of the viral nucleocapsid, in the blood also confirms active infection. Assays for core Ag have been developed with good performance characteristics. The main limitation of core Ag testing is the slightly reduced sensitivity; that is, most assays have a threshold of detection equivalent to ~10,000 IU/mL of HCV RNA.[11] More than 95% of individuals with chronic

TABLE 2. PROS AND CONS OF DIFFERENT HCV DIAGNOSTIC TOOLS

Test/Approach	Pros	Cons	Preferred Settings
Reflex HCV RNA using serum	• Ensure HCV RNA performed on all HCV Ab-positive samples	• RNA degradation • Sample handling within and/or between laboratories • Cost	• Hospital laboratories • Public health/central laboratories • Birth cohort testing
DBS	• Allows for reflex testing • No need for venipuncture • Simple collection—fingerprick (peer testers) • Stable for transport	• Not rapid • Requires centralized laboratory facilities • Reduced sensitivity of HCV RNA (~2 log IU/mL)	• Rural/remote • People who use drugs • Testing drives • Prison at intake • Emergency department (?)
POC Ab test	• Rapid (~20 minutes) • Fingerprick (no venipuncture) • Simple—minimal training	• No reflex HCV RNA	• Screening drives • Emergency department (?)
POC RNA test	• Rapid (~90-100 minutes) • No venipuncture • Easy to administer • Modules available in many low-income countries	• Requires module for reading • Not truly POC (90-100 minutes) • Lower sensitivity than serum PCR	• Very high prevalence settings—opioid substitution clinics • Prison
Core antigen	• Cheaper than RNA • Stable (protein versus RNA)	• Not rapid • Less sensitive than HCV RNA by PCR	• Resource-limited settings

Question marks indicate uncertainty.

HCV have viral levels above this threshold, but there is a small but real risk for missing those with low-level viremia. The advantage of core Ag is primarily lower cost and possibly improved stability, given that it measures a protein rather than RNA. However, core Ag testing also requires a centralized laboratory and is not currently a rapid test. If POC core Ag test could be developed at low cost, it could potentially obviate the need for Ab testing at all, which would greatly simplify testing, particularly in resource-limited settings.

WHICH APPROACH IN WHICH SETTING?

There are pros and cons to the various approaches to HCV diagnosis that make certain strategies preferred in particular clinical settings. The cost of the test is not the only consideration. Changes in sensitivity and/or acceptability may markedly affect overall cost-effectiveness of testing strategies. Other points that must be considered are the availability and need for a centralized laboratory (e.g., DBS, core Ag versus POC test), feasibility of providing results and counseling (e.g., POC testing in emergency department), and acceptability of test/tester to affected populations (e.g., venipuncture challenges for people who inject drugs, use of peer testers with DBS). Optimal settings for the different strategies are highlighted in Table 2.

THE FUTURE

With incredibly effective and very simplified HCV therapy, it is conceivable to imagine a scenario in which a person gives a drop of blood for rapid diagnosis of HCV followed by immediate treatment and a second drop of blood after therapy for confirmation of cure. Hopefully, the new technologies to realize this paradigm will be available in the near future. Until then, clinicians should work with their laboratory partners to streamline the diagnostic cascade using existing tools. Different solutions may work better in different clinical settings, but simplification, particularly the use of reflex RNA testing for Ab-positive samples, will be critical if we are going to reach the ambitious global elimination targets by 2030.

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REFERENCES

1) Centers for Disease Control and Prevention. Testing for HCV infection: An update of guidance for clinicians and laboratorians. MMWR Morb Mortal Wkly Rep 2013;62:362-365.

2) Scott N, Doyle JS, Wilson DP, et al. Reaching hepatitis C virus elimination targets requires health system interventions to enhance the care cascade. Int J Drug Policy 2017;47:107-116.

3) Mera J, Vellozzi C, Hariri S, et al. Identification and clinical management of persons with chronic hepatitis C virus infection—Cherokee Nation, 2012-2015. MMWR Morb Mortal Wkly Rep 2016;65:461-466.

4) Gale HB, Dufour DR, Qazi NN, Kan VL. Comparison of serial hepatitis C virus detection in samples submitted through serology for reflex confirmation versus samples directly submitted for quantitation. J Clin Microbiol 2011;49:3036-3039.

5) Smit PW, Sollis KA, Fiscus S, et al. Systematic review of the use of dried blood spots for monitoring HIV viral load and for early infant diagnosis. PLoS One 2014;9:e86461.

6) Lange B, Cohn J, Roberts T, et al. Diagnostic accuracy of serological diagnosis of hepatitis C and B using dried blood spot samples (DBS): Two systematic reviews and meta-analyses. BMC Infect Dis 2017;17(Suppl. 1):700.

7) Lange B, Roberts T, Cohn J, et al. Diagnostic accuracy of detection and quantification of HBV-DNA and HCV-RNA using dried blood spot (DBS) samples—A systematic review and meta-analysis. BMC Infect Dis 2017;17(Suppl. 1):693.

8) Pallarés C, Carvalho-Gomes Â, Hontangas V, et al. Performance of the OraQuick hepatitis C virus antibody test in oral fluid and fingerstick blood before and after treatment-induced viral clearance. J Clin Virol 2018;102:77-83.

9) Grebely J, Lamoury FMJ, Hajarizadeh B, et al. Evaluation of the Xpert HCV Viral Load point-of-care assay from venepuncture-collected and finger-stick capillary whole-blood samples: A cohort study. Lancet Gastroenterol Hepatol 2017;2:514-520.

10) European Association for the Study of the Liver. EASL recommendations on treatment of hepatitis C 2018. J Hepatol 2018;69:461-511.

11) Freiman JM, Tran TM, Schumacher SG, et al. Hepatitis C core antigen testing for diagnosis of hepatitis C virus infection: A systematic review and meta-analysis. Ann Intern Med 2016;165:345-355.